REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested.

By the foregoing amendment, the specification has been amended to further clarify Applicants' invention. Specifically, the descriptions of Figures 1 and 2 in the Brief Description of the Drawings have been amended to recite the appropriate sequence identifiers, and the descriptions of Figures 7-9 have been amended to fully describe each figure associated with the figure numbers. Support for the amendments can be found in Figures 1 and 2 and in Examples 5-7 on pages 20-22 of the specification, respectively. Further, the specification has been amended to insert the attached substitute Sequence Listing after the last page of the specification (page 27) and the first page of the claims (page 28). Please amend the page numbers accordingly. The substitute Sequence Listing includes the nucleotide sequences recited in Figures 1 and 2. As required, a substitute copy of the Sequence Listing on computer readable form and a Declaration Pursuant to 37 C.F.R. §§ 1.821-.825 are also enclosed herewith. In addition, the specification has been amended, as requested by the Examiner, to comply with 35 U.S.C. § 112, first paragraph. Specifically, the specification has been amended such that it recites full, clear, concise and exact terms.

Claims 1-5, 7, 10-12, 14-15 and 17-22 have been amended to further clarify Applicants' invention. Support for the amendments can be found throughout the

specification. It is noted that none of these amendments are intended to narrow the scope of any element of the claims. Accordingly, no new matter has been added.

I. Formal Matters

Information Disclosure Statement

In the Official Action, the Examiner has noted that the Ferrin et al. reference submitted in the Information Disclosure Statement filed on January 22, 2001 had figure 2 and several claims omitted. Applicants apologize for the inadvertent omission and provide herewith a second copy of Ferrin et al.

Sequence Compliance

The Examiner has stated that Figures 1 and 2 contain sequences which fail to recite the appropriate sequence identifiers. Applicants have amended the description of Figures 1 and 2 to recite the appropriate sequence identifiers. In addition, Applicants have included these sequences recited in Figures 1 and 2 in the substitute sequence listing submitted herewith.

Figures

The Examiner has stated that the explanations for Figures 7-9 are incomplete. Applicants have amended the specification to fully describe each of the figures associated with each Figure number 7-9.

II. Objections

The specification has been objected to for allegedly not complying with 35 U.S.C. § 112, first paragraph. Applicants respectfully traverse this objection.

In order to expedite prosecution in the subject application and not acquiesce to the Examiner's objection, Applicants have amended the specification, as requested by the Examiner, to comply with 35 U.S.C. § 112, first paragraph. Specifically, the specification has been amended such that it recites full, clear, concise and exact terms.

Claims 2 and 21 have been objected to for informalities. Specifically, claim 2 has been objected to because the term "complex" is misspelled, and claim 21 has been objected to for reciting the wrong verb tense. Applicants have amended claim 2 to provide the correct spelling of "complex." With respect to claim 21, Applicants have reviewed claim 21 and cannot find a verb in the wrong tense. Applicants respectfully request that the Examiner indicate the exact location of the incorrect verb tense.

Accordingly, Applicants respectfully request withdrawal of these objections under 35 U.S.C. § 112, first paragraph.

III. Double Patenting Rejections

Statutory Type Double Patenting

Claim 1 has been provisionally rejected under 35 U.S.C. § 101 as allegedly claiming the same invention as that of claim 1 of co-pending Application Serial No.

09/549,949. Applicants respectfully request that the Examiner hold this rejection in abeyance until subject matter in the present application is deemed allowable.

Non-Statutory Double Patenting

Claims 2-9 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-11 of co-pending Application Serial No. 09/549,949 in view of Ferrin et al. Applicants respectfully request that the Examiner hold this rejection in abeyance until subject matter in the present application is deemed allowable.

IV. Rejections Under 35 U.S.C. § 102

Claims 1-11 and 13-19 have been rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Ferrin et al. (WO 97/04111). Applicants respectfully traverse this rejection.

It is well settled law that to anticipate a claim, a single reference must teach each and every element of the claim, and the single reference must be enabling. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.,* 802F.2d 1367, 1379, 231 U.S.P.Q. 81, 90 (Fed. Cir. 1986); *Atlas Powder Co. v. E.I du Pont De Nemours & Co.*, 750 F.2d 1569, 1574, 224 U.S.P.Q. 409, 411 (Fed. Cir. 1984).

Ferrin et al. discloses a method for selectively cloning a DNA fragment of interest using a homologous recombination protein. Ferrin et al. further discloses that during such

cloning, a three-stranded DNA is formed in the presence of the homologous recombination protein.

Applicants submit that the teachings of Ferrin et al. are very different from the present invention. In the present invention, the homologous recombinant protein acts on the two DNA ends that are to be ligated, and the homologous recombination protein is used during DNA ligation to maintain or stabilize the triplex.

To the contrary, in Ferrin et al., the homologous recombination protein acts on the end of the DNA of interest that is to be cloned, and the homologous recombination protein is used with the purpose of protecting the protruding structure (i.e., the cohesive end) of the single stranded DNA at the end of the DNA of interest.

Because the homologous recombination protein is used differently in the present invention when compared to Ferrin et al., the DNA forming the triplex in the presence of the homologous recombination protein is also different. Specifically, in Ferrin et al., the three-stranded structure is formed by one of the two DNA ends that are ligated and an oligonucleotide, whereas in the instant invention, the three-stranded structure is formed by the two DNA ends of double-stranded DNA molecules (not an oligonucleotide) that are ligated. See Figure 1 of the present invention and page 3, lines 15-22 of Ferrin et al.

Furthermore, the two DNA ends ligated in Ferrin et al. are both single-stranded ends, whereas in the present invention, one is a single-stranded end, and the other is a double- stranded end (blunt end).

Therefore, the teachings of Ferrin et al. and the present inventions are very different. In particular, there are differences in i) the way the homologous recombination protein is used; ii) the location on the DNA where the homologous recombination protein acts to form the triplex; and iii) the structure of the two DNA ends that are ligated.

Moreover, the DNA to be ligated in claims 5 and 6 of the instant invention feature an origin of replication, which enables the conversion of a triplex into double-stranded DNA with no nicks after introducing the DNA comprising the triplex into cells and allowing replication. Ferrin et al. neither describes or suggests this feature.

Thus, because Ferrin et al. does not teach each and every element of the claimed invention and because Ferrin et al. is not enabling, this reference cannot and indeed does not anticipate the claimed invention. Therefore, Applicants respectfully request withdrawal of the rejection of claims 1-11 and 13-19 under 35 U.S.C. § 102(b).

V. Rejections Under 35 U.S.C. § 103

Claim 1 has been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Williams et al. in view of Camerini-Otero et al. (A) (*Annu. Rev. Gen.*, 29:509-52, 1995) and Camerini-Otero et al. (B) (U.S. Patent No. 5,460,941). Applicants respectfully traverse this rejection.

In proceedings before the Patent and Trademark Office, the Examiner bears the burden of establishing a *prima facie* case of obviousness based upon the prior art. The Examiner can satisfy this burden by showing, first, that the cited prior art coupled with the

general knowledge at the time of the invention must contain some suggestion or incentive to motivate a skilled artisan to modify or combine references. *See In re Fine,* 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988); *In re Skinner*, 2 U.S.P.Q.2d 1788, 1790 (Bd. Pat. App. & Int. 1986). Second, the Examiner must show that the modification or combination of prior art references must have a reasonable expectation of success (at the time of the invention). *See Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1209, 18 U.S.P.Q.2d 1016, 1023 (Fed. Cir. 1991). Lastly, the Examiner must show that the cited or combined references teach each and every limitation of the claims. *See In re Zurko,* 111 F.3d 887, 888-89, 42 U.S.P.Q.2d 1476, 1478 (Fed. Cir. 1997); *In re Wilson,* 424 F.2d 1382, 1385, 165 U.S.P.Q. 494, 496 (C.C.P.A. 1970).

Camerini-Otero et al. (A) and (B) and Williams et al. teach that the homologous recombination protein protects DNA from nuclease degradation. Camerini-Otero et al. (A) and (B) also disclose a method for selectively cleaving double-stranded DNA by forming a triplex in the presence of a homologous recombination protein.

However, in claim 1 of the present invention, the homologous recombination protein is used with the objective of maintaining (stabilizing) the triplex structure by reacting the protein with the two DNA ends that are ligated, whereas in Camerini-Otero et al. (A) and (B) and Williams et al., the homologous recombination protein is used with the aim of protecting the DNA region against nuclease degradation or methylation by methylase as a result of triplex formation.

Because of this difference in the use of the homologous recombination protein, the DNA forming the triplex in the presence of the homologous recombination protein is different when comparing the present invention with the teachings of the cited references. Specifically, in the cited references, the DNA forming the triplex is the cleaved or degraded DNA and an oligonucleotide used for protecting said DNA, and in the instant invention, the DNA forming the triplex is the ends of the two double-stranded DNAs that are ligated.

Therefore, the teachings of Camerini-Otero et al. (A) and (B) and Williams et al. are very different from the teachings of the present invention. In particular, there are differences in the use of the homologous recombination protein, the DNA on which the homologous recombination protein acts, where the triplex is formed and the structure of the two DNA ends that are ligated.

Because the combination Camerini-Otero et al. (A) and (B) and Williams et al. does not teach each and every element of the claimed invention and because there is no suggestion or incentive in Camerini-Otero et al. (A) or (B) or Williams et al. to motivate a skilled artisan to modify or combine these references, these publication, singly or in combination, do not render the claimed invention obvious.

Therefore, Applicants respectfully request withdrawal of the rejection of claim 1 under 35 U.S.C. § 103(a).

Claims 12, 21-22 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Ferrin et al. Applicants respectfully traverse this rejection.

The differences between the instant invention and Ferrin et al. have been outlined above.

Electroporation is a well known method for introducing DNA into cells. However, according to claim 12 of the present invention, electroporation is employed to introduce DNA into cells for the specific purpose of converting the triplex of the invention into double-stranded DNA within cells. There is no motivation or suggestion or teaching in Ferrin et al. or in the art to lead the skilled artisan to use electroporation to convert a triplex into a double-stranded DNA molecule. Therefore, the fact that electroporation is a method known in the art for introducing DNA into a cell does not effect the patentability of the present invention.

With regard to the kit of claims 21-22, the elements of the kits are double stranded DNA having single-stranded ends and two types of primers. The PCR product produced by these primers is a double-stranded DNA structure having double-stranded ends (blunt ends). This double-stranded DNA structure having double-stranded ends is ligated with a double-stranded DNA having single-stranded ends in the kits of the invention. However, as described above, due to essential differences, Ferrin et al. fails to describe or suggest that one of the two DNA ends used in the ligation reaction should be a single-stranded end of a double-stranded DNA molecule and that the other should be a double-stranded end of a double-stranded DNA molecule.

Thus, because Ferrin et al. does not teach each and every element of the claimed invention and because there is no suggestion or incentive in Ferrin et al. to motivate a

skilled artisan to modify this reference to obtain the present invention, Ferrin et al. does not render the claimed invention obvious.

Therefore, applicants respectfully request withdrawal of the rejection of claims 12, 21-22 under 35 U.S.C. § 103(a).

VI. Rejections Under 35 U.S.C. § 112, second paragraph

Claims 1-22 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants respectfully traverse this rejection.

Claims 1 and 2 allegedly contain circular language rendering them difficult to understand. In order to expedite prosecution in the subject application and not acquiesce to the Examiner's rejection, Applicants have amended claims 1 and 2 to clarify Applicants' invention, thus, rendering this portion of the rejection moot.

Claim 1 has been rejected for reciting the phrase "contacting, under the presence of . . . ." The Examiner has stated that the term "under" connotes location. Applicants submit that the skilled artisan would understand that the reaction takes place "in" the presence of the homologous recombination protein. However, in order to expedite prosecution in the subject application and not acquiesce to the Examiner's rejection, Applicants have amended claim 1 to recite "contacting, in the presence of . . . ." instead of "contacting, under the presence of . . . ."

Claims 1-22 have been rejected for reciting "double-stranded regions," "double-stranded region end," "double-stranded DNA," "double-stranded nucleotide region," and "double-stranded DNA segment." The Examiner has stated that it is unclear whether all these terms refer to the same aspect of Applicants' invention or are entirely different. In order to expedite prosecution in the subject application and not acquiesce to the Examiner's rejection, Applicants have amended claims 1-22 to clarify Applicants' invention, thus, rendering this portion of the rejection moot.

Claims 1-19 have been rejected for reciting the terms "three-stranded structure" and "three-stranded structural portions." The Examiner has stated that the use of these terms is unclear. In order to expedite prosecution in the subject application and not acquiesce to the Examiner's rejection, Applicants have amended claims 1-19 to clarify Applicants' invention, thus, rendering this portion of the rejection moot.

Claim 5 has been rejected for reciting "one DNA." The Examiner has stated that the use of this term is unclear. In order to expedite prosecution in the subject application and not acquiesce to the Examiner's rejection, Applicants have amended claim 5 to clarify Applicants' invention, thus, rendering this portion of the rejection moot.

Claims 5, 20 and 21 have been rejected for reciting the phrase "capable of." The Examiner has stated that this phrase renders the claims indefinite. In order to expedite prosecution in the subject application and not acquiesce to the Examiner's rejection, Applicants have amended claims 5, 20 and 21 to clarify Applicants' invention, thus, rendering this portion of the rejection moot.

Claim 9 has been rejected for reciting the phrase "nucleoside triphosphate or a derivative thereof." The Examiner has stated that the metes and bounds with regard to "derivatives thereof" are unclear. Applicants respectfully traverse this rejection.

Applicants submit that the skilled artisan will recognize and understand that the phrase "nucleoside triphosphates and derivatives thereof" means other nucleoside triphosphates which are obtained from a parent nucleoside triphosphate and still contain the essential elements of the parent nucleoside triphosphate necessary to function as required by the method of the invention.

Claim 19 has been rejected for reciting the term "sandwiched." Because claim 19 does not recite the term "sandwiched," Applicants believe this rejection was intended for claim 20. The Examiner has stated that the use of this term renders the claim vague and indefinite. In order to expedite prosecution in the subject application and not acquiesce to the Examiner's rejection, Applicants have amended claim 20 to clarify Applicants' invention, thus, rendering this portion of the rejection moot.

It is noted that none of the amendments to the claims in response to the 35 U.S.C. § 112, second paragraph, rejections are intended to narrow the scope of any element of the claims.

Therefore, applicants respectfully request withdrawal of the rejection of claims 1-22 under 35 U.S.C. § 112, second paragraph.

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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Date: July 23, 2001

Attachment to Amendment dated July 23, 2001
Marked-up Specification

Marked-up paragraph on page 1, lines 17-21.

--On the other hand, examples for the methods for ligating at a specific site are[, ligation] DNA ligase-mediated ligations[, using DNA ligase,] of a cohesive end of a DNA fragment cleaved by a restriction enzyme, [when appropriate,] or a blunt-end of a DNA fragment [as-it-is,] or [said] a blunt-end to which a suitable adapter has been added.--

Marked-up paragraph beginning on page 2, line 33, to page 3, line 5.

--Therefore, the present invention relates to a method of ligating the ds region end of a double-stranded DNA and the ss region end of [a] another double-stranded DNA[, the]. The method [comprising] comprises contacting, under the presence of a homologous recombinant protein, the ss region end of a double-stranded DNA [having a single-stranded region (ss region) end,] and the ds region end of the other double-stranded DNA [having a double-stranded region (ds region) end comprising] which comprises a sequence that is homologous to the abovementioned ss region nucleotide sequence to form a three-stranded DNA structural complex.--

Marked-up paragraphs on page 3, lines 24-27.

--Figure 1 shows a diagram of the reaction of the DNA cloning method by the Rec A three-strand formation reaction. DNA1 (SEQ ID NO:11) and DNA2 (SEQ ID NO:12) react according to the method of the invention to produce DNA(1+2) (SEQ ID NO:13).

Figure 2 shows a diagram of the 5'RACE reaction by the Rec A three-strand formation reaction. cDNA1 and cDNA 2 react according to the method of the invention to produce cDNA fragment 1+2. cDNA1 contains a small stretch of 10 polyA nucleotides (SEQ ID NO:14).--

Marked-up paragraphs on page 4, lines 5-16.

--[Figure 7 shows a photograph] Figures 7A and 7B show photographs of the gel electrophoretic pattern obtained in Example 5, in which the dependency of each reactant in the DNA end three-strand formation reaction using oligonucleotides was examined. Figure 7A shows an autoradiograph of the ethidium bromide stained agarose gel in 7B.

[Figure 8 shows a photograph] Figures 8A and 8B show photographs of the gel electrophoretic pattern obtained in Example 6, in which the sequence-orientation of oligonucleotides in the DNA end three-strand formation reaction using oligonucleotides, was examined. Figure 8A shows an autoradiograph of the ethidium bromide stainded agarose gel in 8B.

[Figure 9 shows a photograph] Figures 9A-9D show photographs of the gel electrophoretic pattern obtained in Example 7, in which the heat stability of oligonucleotide

sequence in the DNA end three-strand formation reaction, was examined. Figure 9A shows an autoradiograph of the ethidium bromide stained gel in Figure 9B. In Figures 9A and 9B, a labeled 60mer oligonucleotide was used. Figure 9D shows an autoradiograph of the ethidium bromide stained gel in Figure 9C. In Figures 9C and 9D, a labeled 40mer oligonucleotide was used.--

Marked-up paragraph on page 5, lines 15-30.

--The present invention features a method of ligating the ss region end of a double-stranded DNA [comprising a single-stranded region (ss region) end,] and the ds region end of [a] another double-stranded DNA [comprising a double-stranded region (ds region) end having] which comprises a sequence that is homologous to the nucleotide sequence of the aforementioned ss region (therefore, the other strand is complementary to said nucleotide sequence). This ligation can be presumed to occur simultaneously or sequentially at one point or at several positions in a linear DNA molecule, although ligation at two points is preferred. A circular or linear DNA ligation is formed when ligation occurs at one point or several points in each end of one or several DNAs. Although it is not restricted, the formation of a circular DNA ligate is especially preferable. DNA ligates, also named as DNA constituents or DNA recombinants in this specification, means those constructed by a single or several double-stranded DNAs.--

Marked-up paragraph on page 6, lines 17-33.

--Preferable are ligations between two kinds of DNA like those of the above-mentioned (ii) to (iii). In this case, the two ds region ends existing on DNA 1 and/or DNA 2 may have the same or different nucleotide sequences, and similarly, the two ss region ends may have the same or different nucleotide sequences corresponding respectively to the aforementioned ds region ends. "Corresponding respectively" means that a ds region end and an ss region end to be ligated, are in such a relationship that allows the formation of a three-stranded structure according to the present invention. Namely, it means that the nucleotide sequences of the sense-chain of the ds region end to be ligated, and the sense chain of the ss region end are homologous. [(for] For example, the chain corresponding to the single-stranded DNA upstream the double-stranded DNA of Figure 1, is named in this manner (i.e., sense chain) for convenience. Also the other chain down stream is named antisense chain[) of the ds region end to be ligated, and the sense chain of the ss region end (used in the same meaning as the above) are homologous]. As for being "homologous" refer to the prior definition.--

Marked-up paragraph beginning on page 8, line 19, to page 9, line 15.

--In the present invention, the aforementioned ds region end and ss region end are contacted under the presence of a suitable homologous recombinant protein within a liquid medium. The source of such a homologous recombinant protein (or a multi-functional protein that is involved in general recombination) is not questioned, and any protein may be

used, as long as the aforementioned ds region end and ss region end can form a stable complex via said protein, when the said protein is present. Specific examples of such homologous recombinant proteins are, the Rec A protein of *E.coli* origin, multi-functional proteins encoded by the Rec A gene in heat-resistant bacteria (*Thermus thermophillus*) and other enteral bacteria, and already known Rec A-like proteins of *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Methylophilus methylotrophus*, *Vibrio cholerae*, *Ustilago maydis* and such origin. Yeast (*Saccharomyces cerevisiae*) and human-derived Rec-A like proteins are also encompassed in the aforementioned homologous recombinant protein. In the aspects of acquirability, stability and functioning, the *E.coli*-derived Rec A protein or a protein having a similar function [(for example, a modified protein of said protein origin or a fragment thereof)] is preferable for usage. For example, a modified protein of said protein origin or a fragment thereof can be used. As a modified protein, one that is a Rec A gene product created by site-specific mutagenesis of Rec A gene and such, and also comprising the amino acid sequence of the Rec A protein, in which one or more amino acids are deleted, replaced or added, and having a function equivalent to the Rec A protein, to form a complex comprising the aforementioned three- stranded DNA portion, can be given. As a modified protein, with several amino acid deletions, a peptide or protein comprising the binding-domain to single-stranded DNA of Rec A protein, can be given. Examples of such peptides are, those given in the paper of Voloshin et al., Science, Vol. 272, 1996: 868-872. As understood by the above, the word "protein" in the present invention is used as a definition that encompasses peptides as well.--

Marked-up paragraph on page 10, lines 10-14.

--The "contact" according to the present invention can be completed by incubating the mixture prepared as mentioned above at 4 to 54 , preferably for 15 min at about 37 , generally for 30 min. As a result, a DNA ligate (or DNA [constitute] constituent) comprising at least one three-stranded structural portion is formed.--

Marked-up paragraphs beginning on page 10, line 37, to page 11, line 30.

--The method of introduction can be suitably selected according to the starting material (vector) of the vector-derived DNA used as one of the DNAs. For example, when selecting an vector capable of auto-replicating inside *E.coli* as a vector or plasmid, and *E.coli* as the competent cell, introduction of the DNA constituent can be done using method such as electroporation method and calcium-treatment method. By culturing cells into which the above DNA constituent has been introduced, a circular DNA constituent can be amplified, in which the above-mentioned three-stranded structural region (when present) had been converted to double-stranded structural region. [(this] This structural region is presumed to be in a state in which the nucleotide sequence corresponding to the above-mentioned ss region end has been removed from the abovementioned ds region end, and in which the ds region end and the ss region end is covalently-linked via a phosphodiester bond[)].

If ordinary methods used to introduce a gene into the aforementioned cells are followed, a circular DNA constituent will be more efficiently introduced into a cell than a

linear DNA constituent. Therefore, when applying the method of the present invention using a PCR product [(which is obtained by a PCT that uses as a primer, an oligonucleotide comprising nucleotide sequences corresponding respectively to the two types of ss region ends followed by each of the nucleotide sequences of the 5 or 3 end region of the gene to be ligated, and uses said gene as a template)] as the DNA having the ds region end, those byproducts that are not correctly elongated by PCR will not be introduced to cells, since they do not form circular DNA constituents. The above-mentioned PCR product is obtained by a PCR reaction that uses as a primer, an oligonucleotide comprising nucleotide sequences corresponding respectively to the two types of ss region ends followed by each of the nucleotide sequences of the 5 or 3 end region of the gene to be ligated, and uses said gene as a template. Therefore, only the PCR products resulting from the correct amplification of the gene to be cloned by PCR, can be obtained by the method of the present invention.--

Marked-up paragraph on page 12, lines 12-20.

--Examples of methods for converting a three-stranded structure to a [double-strand ed] double-stranded structure are, (1) the method that comprises transfecting the nucleic acid ligate obtained by the three-strand formation reaction into a prokaryotic or eukaryotic cell, and converting the nucleic acid-ligated region including the three-strand structure into double-stranded DNA, or (2) the method of converting the nucleic acid-ligated region

including the three-strand structure into double-stranded DNA using nucleic-acid modification enzymes, within a test tube.--

Attachment to Amendment dated July 23, 2001
Marked-up Claims

1. (Amended) A method of ligating a double-stranded [region (ds region)] end of a double-stranded DNA and a single-stranded [region (ss region)] end of [a] another double-stranded DNA, the method [comprising] comprises contacting, [under] in the presence of a homologous recombinant protein, the [ss region] single-stranded end of [a] said other double-stranded DNA [having an ss region end,] and the [ds region] double-stranded end of [a] said double-stranded DNA [having a ds region end comprising], said double-stranded DNA comprises a sequence that is homologous to the nucleotide sequence of said [ss region nucleotide sequence] single-stranded end to form a three-stranded DNA structural complex comprising said [ss region] single-stranded end and said [ds region] double-stranded end.

2. (Amended) The method of ligation of claim 1, wherein said three-stranded DNA structural [comples] complex is a circular DNA complex having a three-stranded structure in two positions, wherein said three-stranded structure is made by the ligation of:

a) a double-stranded DNA comprising [an ss region end] a single-stranded region at both ends, and

b) a double-stranded DNA having at both ends[, ds region ends] a double-stranded region comprising sequences that are respectively homologous to [the above mentioned ss] said single-stranded nucleotide regions in a). [, or the ligation

of a double-stranded DNA comprising an ss region end and a ds region end, and a double-stranded DNA comprising a ds region end having a sequence that is homologous to the nucleotide sequence of the above mentioned ss nucleotide region and an ss region end comprising a sequence that is homologous to the nucleotide sequence of the above mentioned ds nucleotide region]; or said three-stranded structure is made by the ligation of:

c) a double-stranded DNA comprising a singe-stranded region at one end and a double-stranded region at the other end, and

d) a double-stranded DNA comprising a double-stranded region at one end having a sequence that is homologous to the nucleotide sequence of said single-stranded nucleotide region in a) and a single-stranded region at the other end comprising a sequence that is homologous to the nucleotide sequence of the double-stranded nucleotide region in a).

3. (Amended) The method of ligation of claim 2, wherein the nucleotide sequences of the two [ss] single-stranded regions in a) are mutually non-complementary.

4. (Amended) The method of ligation of claim 2, wherein the two [ss] single-stranded region ends in a) are within the same double-stranded DNA.

5. (Amended) The method of ligation of claim 2, wherein one DNA from a) and b) or one DNA from c) and d) [is capable of conferring] confers the ability of auto-replicating within competent cells.

7. (Amended) The method of ligation of claim 1, wherein the nucleotide sequence of the [ss] single-stranded region is at least a 6mer.

10. (Amended) The method of ligation of claim 1, [further comprising] wherein said method comprises a step of converting the three-stranded structure formed to a double-stranded structure.

11. (Amended) The method of ligation of claim 10, wherein the conversion of the three-stranded structure to a double-stranded structure is done by inserting [a] the DNA complex comprising a three-stranded structure into cells.

12. (Amended) The method of ligation of claim 11, wherein the insertion of [a] the DNA complex comprising a three-stranded structure into cells is done by electroporation.

14. (Amended) The method of ligation of claim 1, [further comprising] wherein said method further comprises steps of converting the three-stranded [structural

portion] structure into a double-stranded structure by treating the DNA complex having the three-stranded structure with endonuclease [in advance, and then], inserting [this] said treated DNA complex into cells, and culturing the transformant thus obtained to amplify DNA.

15. (Amended) A DNA constituent comprising at least one three-stranded [structural portion] structure comprising a single-stranded region [(ss region) end] and a double-stranded region [(ds region) end comprising] which comprises a sequence that is homologous to said [ss] single-stranded region [nucleotide sequence].

17. (Amended) The DNA constituent of claim 15, wherein the constituent comprises two three-stranded [structural portions] structures, and the [ss region] single-stranded nucleotide sequences forming these [structural portions] structures are mutually non-homologous.

18. (Amended) The DNA constituent of claim 15, wherein each [ss] single-stranded region nucleotide is at least 6 mer.

19. (Amended) The DNA constituent of claim 15, wherein the three-stranded [structural portion] structure forms a complex with a homologous recombinant protein.

20. (Amended) The DNA constituent of claim 15, wherein one double-stranded DNA segment [sandwiched] which is between two three-stranded [structural portions, is capable of conferring] structures confers the ability of auto-replicating within competent cells, and the other double-stranded DNA segment comprises the whole or part of the gene to be cloned.

21. (Amended) A gene-cloning kit comprising the following components:

a) a DNA, which is a double-stranded DNA comprising a single-stranded region [(ss region)] at both ends, wherein the nucleotide sequences of these [ss] single-stranded regions are mutually non-complementary, and furthermore comprises a DNA sequence [capable of conferring] which confers to the double-stranded region of said double-stranded DNA, the ability of auto-replicating within competent cells;

b) an oligonucleotide primer comprising as a part of the [5] 5' end sequence, a sequence that is complementary to the one [ss] single-stranded region nucleotide sequence of (A), and [furthermore,] is complementary to a part of the end of the sequence [of one end] of the gene to be cloned, and;

c) an oligonucleotide primer comprising as a part of the [5] 5' end sequence, a sequence that is complementary to the other [ss] single-stranded region nucleotide sequence of (A), and [furthermore,] is complementary to a part of the

other end of the sequence [of the end, which is different to that in (B),] of the gene to be cloned.

22. (Amended) The kit of claim 21, wherein the nucleotide sequence of each [ss] single-stranded region is at least 6 mer.